PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 37/02 — A2

(11) International Publication Number: WO 91/15222

(43) International Publication Date: 17 October 1991 (17.10.91)

(21) International Application Number: PCT/US91/01861

(22) International Filing Date: 20 March 1991 (20.03.91)

(30) Priority data:
504,495 4 April 1990 (04.04.90) US

(71) Applicant: GENENTECH, INC. [US/US]; 460 Point San Bruno Boulevard, South San Francisco, CA 94080 (US).

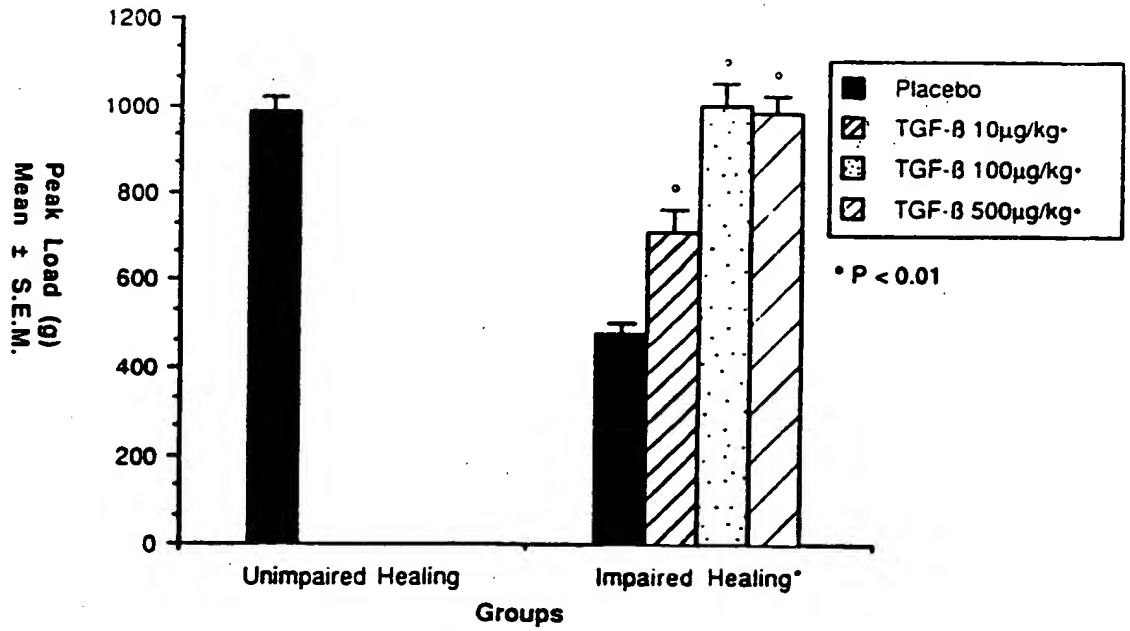
(72) Inventors: AMENTO, Edward, P. ; 1030 Westridge Drive, Portola Valley, CA 94025 (US). BECK, L., Steven ; 1635 Clay Drive, Los Altos, CA 94024 (US).

(74) Agents: HASAK, Janet, E. et al.; Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080 (US).

(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: METHOD OF PREDISPOSING MAMMALS TO ACCELERATED TISSUE REPAIR



(57) Abstract

A method of predisposing a mammal to accelerated tissue repair is provided. This method comprises systemically administering to the mammal, prior to its exposure to tissue damage, an effective amount of TGF-β. Preferably, the TGF-β is administered no more than about 24

Several groups have characterized the latent form of TGF-β1 secreted by human platelets. Pircher et al., *supra*, stated that it has an apparent molecular weight of 400 Kd. More recently, it has been characterized as a three-component complex of about 235 Kd, wherein the active TGF-β1 (25 Kd dimer) is non-covalently associated with the remainder of the processed precursor (75 Kd dimer), which in turn is disulfide-bonded to an unrelated protein of 125-160 Kd (Wakefield et al., J. Biol. Chem., 263, *supra*; Miyazono et al., *supra*; Miyazono et al., J. Cell Biochem. Supp., 0 (12 Part A), 1988, p. 200; Wakefield et al., J. Cell. Biochem. Suppl., 11A: 0, 46 (1987)).

The function of the binding protein of 125-160 Kd remains to be elucidated. Recent characterizations indicate that it contains at least 14 EGF-like repeats and six potential N-glycosylation sites and calcium binding domains (Kanzaki et al., "TGF-β", NY Acad. Sci. meeting abstract, May 18-20, 1989; Miyazono, "TGF-β", NY Acad. Sci. meeting abstract, May 18-20, 1989). Latent TGF-β secreted by many cells in culture has a similar structure (Wakefield et al., J. Biol. Chem., *supra*), and this is the form in which TGF-β1 is probably perceived initially by target cells *in vivo*. It has been suggested that the precursor remainder of TGF-β may have an important independent biological function based on conservation of sequences in the precursor region (Roberts et al., Recent Progress in Hormone Research, *supra*). Additionally, a mutation at position 33 of precursor TGF-β1 is reported to increase the yield of mature TGF-β1, and dimerization of the precursor "pro" region is suggested as necessary to confer latency (Brunner et al., J. Biol. Chem., 264: 13660-13664 (1989)).

Normal repair of tissue is a complex, sequential process involving many cell types. Fibroblasts, inflammatory cells, and keratinocytes all function in an integrated manner to promote cell division, differentiation, and migration. These processes in turn lead to enhanced connective tissue deposition and angiogenesis. Recent data suggest that these processes may be mediated both in an autocrine and paracrine manner by peptide growth factors such as TGF-β (Postlethwaite et al., J. Exp. Med., 165: 251-256 (1987); Assoian et al., Nature, 308: 804-806 (1984)). Levels of endogenous TGF-β have been reported to increase transiently in wound chambers of the rat (Cromack et al., J. Surg. Res., 42: 622-628 (1987)). Also, a crude extract of platelets containing multiple growth factors promoted healing of chronic skin ulcers (Knighton et al., Ann. Surg., 204: 322-330 (1986)). The results of these studies indirectly support the hypothesis that normal healing is mediated by locally produced peptide growth factors.

In vivo, TGF-β1 causes granulation tissue to form when injected intradermally (Roberts et al., Proc. Nat. Acad. Sci. USA, 83: 4167-4171 (1986); Sporn et al., Science, 219: 1329-1331 (1983)). *In vitro*, TGF-β1 stimulates the expression of fibronectin and collagen type I, in part mediated via increased levels of mRNA, and increases the deposition of fibronectin into the pericellular matrix (Wrana et al., Eur. J. Biochem., 159: 69-76 (1986); Ignotz and Massague, J.Biol. Chem., 261: 4337-4345 (1986); Fine and Goldstein, J. Biol. Chem., 262:

3897-3902 (1987); Ignotz et al., J. Biol. Chem., 262: 6443-6446 (1987); Raghow et al., J. Clin. Invest., 79: 1285-1288 (1987); Varga and Jimeniz, Biochem. Biophys. Res. Commun., 138: 974-980 (1986)).

A single application of TGF-β in collagen vehicle to incisions in normal rats significantly increased tensile strength compared with untreated or collagen vehicle treated incisions (Mustoe et al., Science, 237: 1333-1336 (1987)). See also Brown et al., Ann. Surg., 208: 788-794 (1988). In another study it was reported that TGF-β treatment reversed doxorubicin depressed uptake of hydroxyproline and thymidine in wound chambers in rats, suggesting that TGF-β might enhance the strength of the incisions by stimulating proliferation of cells and enhancing collagen synthesis (Grotendorst et al., J. Clin. Invest., 76: 2323-2329 (1985)).

These results were extended using an animal model that more closely approximates healing of surgical incisions (Curtsinger et al., Surgery, Gynecology & Obstetrics, 168: 517-522 (1989)). It was hypothesized that because TGF-β is a potent chemoattractant for human fibroblasts (Postlethwaite et al., *supra*,) and stimulates collagen synthesis in cultures of renal fibroblasts in rats (Roberts et al., Proc. Natl. Acad. Sci. USA, *supra*), it may increase tensile strength by directly stimulating production of collagen by fibroblasts or by attracting inflammatory cells that may release peptide growth factors into the wounded area (Madtes et al., Cell, 53: 285-293 (1988); Morhenn, Immunol. Today, 9: 104-107 (1988)). In addition to the scientific literature, the patent literature has also disclosed that TGF-β is useful in treating existing traumata when administered systemically or applied topically to the traumatized tissue, with promotion of rapid proliferation of cells, particularly fibroblast cells (see, e.g., EP 128,849; EP 105,014; U.S. Pat. Nos. 4,843,063; 4,774,322; 4,774,228; and 4,810,691). There is, however, also a need for an agent that predisposes mammals to accelerated tissue repair before the mammals have been subjected to trauma.

Accordingly, it is an object of the present invention to provide a method for treating mammals that have not yet experienced tissue damage to promote accelerated proliferation of the cells surrounding the traumata and consequently rapid healing.

This object and other objects will become apparent to one of ordinary skill in the art.

Disclosure of Invention

This invention provides a method of predisposing a mammal to accelerated tissue repair comprising systemically administering to the mammal, prior to its exposure to tissue damage, an effective amount of TGF-β. Preferably, the TGF-β is administered no more than about 24 hours prior to the tissue damage exposure. More preferably, the TGF-β is administered within a time range of from about 24 hours to greater than about 5 minutes before exposure to tissue damage.

Surprisingly, it has been found that administration of a single dose of TGF-β systemically to a mammal at least 24 hours in advance of wounding accelerates healing of

the wound dramatically. This discovery was particularly surprising because TGF-β has a circulating half-life in plasma of only about 5 minutes.

Brief Description of the Drawings

Figure 1 depicts the sequences of human TGF-β1, human TGF-β2, human TGF-β3, chick TGF-β4, and frog TGF-β5.

Figure 2 represents the peak load, which is a measure of strength, of linear skin incision wounds. Rats were treated intramuscularly with 5 mg prednisolone (asterisk) at the time of wounding to impair healing processes or treated with saline (black) as an unimpaired-healing control. Saline (diagonal stripes) or 10 μg/kg rhTGF-β1 (cross-hatching) was administered intravenously 24 hours before (-24 hr.) or at the time of (0 hr.) wounding.

Figure 3 represents the peak load of the impaired-healing rat skin linear incision wounds treated intravenously with saline (black) or with 10 μg/kg, 100 μg/kg, or 500 μg/kg of rhTGF-β1 and intramuscularly with 5 mg methylprednisolone at the time of wounding. A group treated with saline but not treated with methylprednisolone served as an unimpaired-healing control.

Description of the Preferred Embodiments

A. Definitions

As used herein, the term "TGF-β" refers to the family of molecules described hereinabove, having the full-length, native amino acid sequence of any of the TGF-βs from any species. Reference to such TGF-β herein will be understood to be a reference to any one of the currently identified forms, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, and TGF-β5 (whose sequences are shown in Figure 1), as well as to TGF-β species identified in the future, including polypeptides derived from the sequence of any known TGF-β and identical at 75% or more of the residues, their alleles, and their predetermined amino acid sequence variants, so long as they are effective in the method described herein. The specific terms "TGF-β1," "TGF-β2," and "TGF-β3" refer to the TGF-βs defined in the literature, e.g., Derynck et al., Nature, *supra*, Seyedin et al., J. Biol. Chem., 262, *supra*, and deMartin et al., *supra*. In addition, the TGF-β is suitably useful in the latent form or as an associated or unassociated complex of precursor and mature TGF-β.

Members of the TGF-β family are defined as those which have nine cysteine residues in the mature portion of the molecule, share at least 65% sequence identity with other known TGF-β sequences in the mature region, and may compete for the same receptor. In addition, they all appear to be encoded as a larger precursor that shares a region of high homology near the N-terminus and shows conservation of three cysteine residues in the portion of the precursor that will later be removed by processing. Moreover, the TGF-βs appear to have a processing site with four or five basic amino acids.

The TGF-β is appropriately from any source, preferably mammalian, and most preferably human. TGF-β from animals other than humans, for example, porcine or bovine

sources, can be used for treating humans. Likewise, if it is desirable to treat other mammalian species such as domestic, farm, zoo, sports, or pet animals, human TGF-β, as well as TGF-β from other species, is suitably employed.

As used herein, the term "tissue damage" refers to any form of damage or trauma to soft or hard tissue, including thermally and/or mechanically induced trauma as well as damage caused by inflammatory, infectious, and immune responses. Examples of tissue damage include surgical incisions, such as internal and epidermal surgical incisions, and corneal surgery; burns, whether first, second, or third degree; bone damage such as bone fractures, bony defects, and prosthetic implants, including injury attendant surgery such as hip replacements; wounds, including lacerations, incisions, and penetrations; sites of expected development of ulcers such as, e.g., diabetic, dental, haemophiliac, varicose, or decubitus ulcers; chronic conditions or ulcers converted to acute wounds, preferably by surgery; infections of the bone such as osteomyolitis; and any inflammatory or immune response of soft tissue such as that seen with rheumatoid arthritis or any inflammatory condition leading to bone loss, whether infectious or non-infectious.

B. Modes for Carrying Out the Invention

The method of this invention involves systemic administration to a mammal, including domestic, farm, zoo, sports, or pet animals, but preferably a human, of an effective amount of TGF-β as an agent that predisposes the tissue to accelerated repair.

The types of patients that may be treated by the method of this invention include not only those who do or would be expected to undergo normal tissue repair, but also those that would be predicted to or do exhibit abnormal tissue repair. Impaired wound healing has many causes, including diabetes, uremia, malnutrition, vitamin deficiencies, and systemic treatment with corticosteroids, radiation, or antineoplastic agents such as doxorubicin. Thus, this invention contemplates treatment of the latter as well as the former types of patients.

The TGF-β molecule will be formulated and dosed in a fashion consistent with good medical practice taking into account the specific tissue involved, the condition of the individual patient, the site of delivery of the TGF-β, the method of administration, and other factors known to practitioners. Thus, for purposes herein, the "therapeutically effective amount" of the TGF-β is an amount that is effective to accelerate tissue repair in a mammal that undergoes tissue damage after administration of the TGF-β.

The TGF-β is prepared for storage or administration by mixing TGF-β having the desired degree of purity with physiologically acceptable carriers, excipients, or stabilizers. Such materials are non-toxic to recipients at the dosages and concentrations employed. If the TGF-β is water soluble, it may be formulated in a buffer such as acetate or other organic acid salt, preferably at a pH of about 5 to 6. If a TGF-β variant is only partially soluble in water, it may be prepared as a microemulsion by formulating it with a nonionic surfactant such as Tween, Pluronics, or polyethylene glycol (PEG), e.g., Tween 80, in an amount of 0.04-0.05% (w/v),

to increase its solubility. Optionally other ingredients may be added such as antioxidants, e.g., ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; chelating agents such as EDTA; and sugar alcohols such as mannitol or sorbitol.

The TGF-β to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). The TGF-β ordinarily will be stored in lyophilized form or as an aqueous solution since it is highly stable to thermal and oxidative denaturation. The pH of the TGF-β preparations typically will be about 5, although higher or lower pH values may also be appropriate in certain instances. It will be understood that use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of salts of the TGF-β.

Therapeutic compositions containing the TGF-β generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Sustained release formulations may also be prepared, and include the formation of microcapsular particles and implantable articles. For preparing sustained-release TGF-β compositions, the TGF-β is preferably incorporated into a biodegradable matrix or microcapsule. A suitable material for this purpose is a polylactide, although other polymers of poly-(α-hydroxycarboxylic acids), such as poly-D-(-)-3-hydroxybutyric acid (EP 133,988A), can be used. Other biodegradable polymers include poly(lactones), poly(acetals), poly(orthoesters), or poly(orthocarbonates). The initial consideration here must be that the carrier itself, or its degradation products, is nontoxic in the target tissue and will not further aggravate the condition. This can be determined by routine screening in representative animal models such as impaired rat skin linear incision models, or, if such models are unavailable, in normal animals.

For examples of sustained release compositions, see U.S. Patent No. 3,773,919, EP 58,481A, U.S. Patent No. 3,887,699, EP 158,277A, Canadian Patent No. 1176565, U. Sidman et al., Biopolymers, 22: 547 (1983), and R. Langer et al., Chem. Tech., 12: 98 (1982).

Tissue damage caused by infections may be treated with TGF-β formulated with an effective amount of an antibiotic such as cepholosporin or penicillin. Alternatively, the antibiotic and TGF-β may be administered separately to the patient using the general methods described above. The treating physician will be able to determine the proper dosages and administration routes of antibiotic based on conventional therapy for treating infectious conditions.

The dosage of TGF-β to be employed is dependent upon the factors described above, especially the type of tissue damage which is expected. As a general proposition, a dose of

about 0.015 to 5 mg/kg, preferably to 0.5 mg/kg, of TGF-β may be administered to the patient, whether via, e.g., one or more single administrations, continuous infusion, or bolus injection. The advantage of this invention lies in the use of only one administration of TGF-β, preferably intravenously, so one dose is preferred. However, other dosage regimens may be useful. This administration takes place prior to infliction of damage to the tissue, e.g., before surgery, preferably no more than about 24 hours before tissue damage is inflicted, and more preferably from within 24 hours to greater than about 5 minutes prior to tissue damage.

The invention is more fully illustrated in the example set forth below, which is intended to represent one embodiment of the invention, but not the only embodiment.

EXAMPLE I

Material: Recombinant human TGF-β1 was cloned (Derynck et al., Nature, *supra*) and expressed in Chinese hamster ovary cells (using a method such as that described by Graycar et al., Molecular Endocrinology, 3: 1977-1986 (1989) and U.S Pat. No. 4,886,747 issued December 12, 1989). The protein was purified by harvesting the cell culture fluid, concentrating this fluid with a Pellicon cassette system, diluting the concentrate with three vols. of a mixture of 50:1 of reagent alcohol to HCl, allowing the mixture to sit for 1 hour at 4°C, adjusting the pH to 7.5-8, centrifuging the mixture, loading the supernatant on a cation exchange S Sepharose Fast Flow column (previously equilibrated with 6 M urea, 20 mM MOPS buffer, pH 8), washing the column with the same buffer, eluting with a gradient of 0 to 0.4 M sodium chloride in the same buffer, making a pool from the gradient fractions run on a gel, adjusting the pH of the pool to 4.5, applying the pool to a second cation exchange SP Toyopearl column previously equilibrated in 2 M urea, 50 mM sodium acetate buffer at pH 4.5, washing the column with the same buffer, eluting with a gradient of 0 to 1 M sodium chloride in the same buffer, making a pool from the gradient fractions run on a gel, concentrating the pool on a stirred cell Amicon concentrator, loading the concentrate on a HW55S Toyopearl gel filtration column, washing with 1 M acetic acid, making a pool from the gradient fractions run on a gel, and exchanging the pool into 20 mM sodium acetate buffer at pH 5 over Cellufine GH-25.

Vehicle (saline) was formulated in the sodium acetate buffer at pH 5 without the TGF-β1. The material was stored at 4°C until use.

Animal Surgery: Adult male Sprague Dawley rats, 300-350 grams (Charles River Laboratories, Wilmington, MA), maintained in accordance with guidelines from the NIH and the American Association for the Accreditation of Laboratory Animal Care, were anesthetized by an intramuscular injection of ketamine hydrochloride/xylazine hydrochloride/acetylpromazine maleate mixture. The hair was clipped from the back and sides and disinfected with betadine and 70% alcohol rinse. At this time each rat was given a single intravenous (iv) injection of either saline or one of four concentrations of TGF-β1 at a volume of 1.0 ml/kg. After injection of vehicle or TGF-β1, two pairs of symmetrical

transverse full-thickness skin incisions approximately 2.5 cm in length were made by cutting through the subdermal panniculus carnosus musculature. Each wound was closed with two interrupted 4-0 stainless steel sutures evenly divided across the wound. After surgery each rat was administered either a single intramuscular injection of 5 mg methylprednisolone to inhibit inflammation and thus impair the healing process or saline to serve as an unimpaired healing control. The animals were returned to their cages and allowed to recover.

Additional animals were treated in an identical manner with the exception of a single intravenous dose of TGF-β1 administered 24, 48, or 72 hours before surgery rather than at the time of surgery.

Tissue Sampling: In a time-dependent manner rats were euthanized and 1-2 mm cross-sections of the wound from the center of each scar were removed with samples fixed in 10% neutral buffered formalin for light microscopic examination and Karnovsky's solution for electron microscopy. Two 8 x 25 mm samples from each wound were removed and fixed in 10% formalin for seven days for wound strength determinations.

Tensometry: Tissues were uniformly trimmed in width and length (8 mm x 25 mm) to assure that the edges of the scar were exposed on both sides of the sample. Tensometry was performed on coded samples using a calibrated tensometer (Instron Universal Testing Instrument Model 1011, Instron Corp., Canton, MA). The value determined was breaking strength (g), which is a measure of force in grams applied to the tissue at the point where the scar tissue visually breaks and a major deflection occurs in the tracing.

Results: Two separate studies were performed in which there were an unimpaired-healing control (saline) group and an impaired-healing control (saline) group and TGF-β1-treated group(s). The first study compared the effects of 10 μg/kg TGF-β1 to saline control when administered intravenously either 24 hours prior to or just before skin incision. Results of this study are presented in Figure 2 and indicate that wounds treated with 10 μg/kg TGF-β1 exhibited increased strength ($p < 0.05$) compared to its concurrent vehicle control. In addition, the impaired-healing wounds treated with TGF-β1 were approximately 90% as strong as unimpaired-healing wounds treated with vehicle.

The second study was identical in design with the addition of 100 μg/kg and 500 μg/kg doses of TGF-β1. Results, presented in Figure 3, indicate that all three dose levels of TGF-β1 increased the strength of linear incision wounds compared with impaired-healing vehicle control ($p < 0.01$). Both the 100 and 500 μg/kg doses of TGF-β1 returned impaired-healing wounds to the same strength as unimpaired-healing vehicle treated wounds (Fig. 3).

Thus, TGF-β is effective when administered as single iv doses of 10 to 500 μg/kg in accelerating wound healing in this model. This model is predictive of the results that one would obtain in a clinical trial.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Genentech, Inc.

(ii) TITLE OF INVENTION: Method of Predisposing Mammals to Accelerated Tissue Repair

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Genentech, Inc.
(B) STREET: 460 Point San Bruno Blvd
(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: U.S. Ser. No. 07/504,495
(B) FILING DATE: 4 April 1990

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Hasak, Janet E.
(B) REGISTRATION NUMBER: 28,616
(C) REFERENCE/DOCKET NUMBER: 637

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415/266-1896
(B) TELEFAX: 415/952-9881
(C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 390 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(iv) SEQUENCE DESCRIPTION:SEQ ID NO:1:

```
Met Pro Pro Ser Gly Leu Arg Leu Leu Pro Leu Leu Leu Pro Leu
 1               5                  10                  15

Leu Trp Leu Leu Val Leu Thr Pro Gly Pro Pro Ala Ala Gly Leu
```

20 25 30

Ser Thr Cys Lys Thr Ile Asp Met Glu Leu Val Lys Arg Lys Arg
35 40 45

Ile Glu Ala Ile Arg Gly Gln Ile Leu Ser Lys Leu Arg Leu Ala
50 55 60

Ser Pro Pro Ser Gln Gly Glu Val Pro Pro Gly Pro Leu Pro Glu
65 70 75

Ala Val Leu Ala Leu Tyr Asn Ser Thr Arg Asp Arg Val Ala Gly
80 85 90

Glu Ser Ala Glu Pro Glu Pro Glu Pro Glu Ala Asp Tyr Tyr Ala
95 100 105

Lys Glu Val Thr Arg Val Leu Met Val Glu Thr His Asn Glu Ile
110 115 120

Tyr Asp Lys Phe Lys Gln Ser Thr His Ser Ile Tyr Met Phe Phe
125 130 135

Asn Thr Ser Glu Leu Arg Glu Ala Val Pro Glu Pro Val Leu Leu
140 145 150

Ser Arg Ala Glu Leu Arg Leu Leu Arg Leu Lys Leu Lys Val Glu
155 160 165

Gln His Val Glu Leu Tyr Gln Lys Tyr Ser Asn Asn Ser Trp Arg
170 175 180

Tyr Leu Ser Asn Arg Leu Leu Ala Pro Ser Asp Ser Pro Glu Trp
185 190 195

Leu Ser Phe Asp Val Thr Gly Val Val Arg Gln Trp Leu Ser Arg
200 205 210

Gly Gly Glu Ile Glu Gly Phe Arg Leu Ser Ala His Cys Ser Cys
215 220 225

Asp Ser Arg Asp Asn Thr Leu Gln Val Asp Ile Asn Gly Phe Thr
230 235 240

Thr Gly Arg Arg Gly Asp Leu Ala Thr Ile His Gly Met Asn Arg
245 250 255

Pro Phe Leu Leu Leu Met Ala Thr Pro Leu Glu Arg Ala Gln His
260 265 270

Leu Gln Ser Ser Arg His Arg Arg Ala Leu Asp Thr Asn Tyr Cys
275 280 285

Phe Ser Ser Thr Glu Lys Asn Cys Cys Val Arg Gln Leu Tyr Ile
290 295 300

Asp Phe Arg Lys Asp Leu Gly Trp Lys Trp Ile His Glu Pro Lys
305 310 315

Gly Tyr His Ala Asn Phe Cys Leu Gly Pro Cys Pro Tyr Ile Trp
320 325 330

Ser Leu Asp Thr Gln Tyr Ser Lys Val Leu Ala Leu Tyr Asn Gln
335 340 345

His Asn Pro Gly Ala Ser Ala Ala Pro Cys Cys Val Pro Gln Ala
350 355 360

Leu Glu Pro Leu Pro Ile Val Tyr Tyr Val Gly Arg Lys Pro Lys
365 370 375

Val Glu Gln Leu Ser Asn Met Ile Val Arg Ser Cys Lys Cys Ser
380 385 390

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 414 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(iv) SEQUENCE DESCRIPTION:SEQ ID NO:2:

Met His Tyr Cys Val Leu Ser Ala Phe Leu Ile Leu His Leu Val
1 5 10 15

Thr Val Ala Leu Ser Leu Ser Thr Cys Ser Thr Leu Asp Met Asp
20 25 30

Gln Phe Met Arg Lys Arg Ile Glu Ala Ile Arg Gly Gln Ile Leu
35 40 45

Ser Lys Leu Lys Leu Thr Ser Pro Pro Glu Asp Tyr Pro Glu Pro
50 55 60

Glu Glu Val Pro Pro Glu Val Ile Ser Ile Tyr Asn Ser Thr Arg
65 70 75

Asp Leu Leu Gln Glu Lys Ala Ser Arg Arg Ala Ala Ala Cys Glu
80 85 90

Arg Glu Arg Ser Asp Glu Glu Tyr Tyr Ala Lys Glu Val Tyr Lys
95 100 105

Ile Asp Met Pro Pro Phe Phe Pro Ser Glu His Ala Ile Pro Pro
110 115 120

Thr Phe Tyr Arg Pro Tyr Phe Arg Ile Val Arg Phe Asp Val Ser
125 130 135

Ala Met Glu Lys Asn Ala Ser Asn Leu Val Lys Ala Glu Phe Arg
140 145 150

Val Phe Arg Leu Gln Asn Pro Lys Ala Arg Val Pro Glu Gln Arg
155 160 165

Ile Glu Leu Tyr Gln Ile Leu Lys Ser Lys Asp Leu Thr Ser Pro
170 175 180

Thr Gln Arg Tyr Ile Asp Ser Lys Val Val Lys Thr Arg Ala Glu
185 190 195

Gly Glu Trp Leu Ser Phe Asp Val Thr Asp Ala Val His Glu Trp
200 205 210

Leu His His Lys Asp Arg Asn Leu Gly Phe Lys Ile Ser Leu His
215 220 225

Cys Pro Cys Cys Thr Phe Val Pro Ser Asn Asn Tyr Ile Ile Pro
230 235 240

Asn Lys Ser Glu Glu Leu Glu Ala Arg Phe Ala Gly Ile Asp Gly
245 250 255

Thr Ser Thr Tyr Thr Ser Gly Asp Gln Lys Thr Ile Lys Ser Thr
260 265 270

Arg Lys Lys Asn Ser Gly Lys Thr Pro His Leu Leu Leu Met Leu
275 280 285

Leu Pro Ser Tyr Arg Leu Glu Ser Gln Gln Thr Asn Arg Arg Lys
290 295 300

Lys Arg Ala Leu Asp Ala Ala Tyr Cys Phe Arg Asn Val Gln Asp
305 310 315

Asn Cys Cys Leu Arg Pro Leu Tyr Ile Asp Phe Lys Arg Asp Leu
320 325 330

Gly Trp Lys Trp Ile His Glu Pro Lys Gly Tyr Asn Ala Asn Phe
335 340 345

Cys Ala Gly Ala Cys Pro Tyr Leu Trp Ser Ser Asp Thr Gln His
350 355 360

Ser Arg Val Leu Ser Leu Tyr Asn Thr Ile Asn Pro Glu Ala Ser
365 370 375

Ala Ser Pro Cys Cys Val Ser Gln Asp Leu Glu Pro Leu Thr Ile
380 385 390

Leu Tyr Tyr Ile Gly Lys Thr Pro Lys Ile Glu Gln Leu Ser Asn
395 400 405

Met Ile Val Lys Ser Cys Lys Cys Ser
410 414

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 410 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(iv) SEQUENCE DESCRIPTION:SEQ ID NO:3:

```
Met His Leu Gln Arg Ala Leu Val Val Leu Ala Leu Leu Asn Phe
  1               5                  10                  15

Ala Thr Val Ser Leu Ser Leu Ser Thr Cys Thr Thr Leu Asp Phe
             20                  25                  30

Gly His Ile Lys Lys Lys Arg Val Glu Ala Ile Arg Gly Gln Ile
         35                  40                  45

Leu Ser Lys Leu Arg Leu Thr Ser Pro Pro Glu Pro Thr Val Met
     50                  55                  60

Thr His Val Pro Tyr Gln Val Leu Ala Leu Tyr Asn Ser Thr Arg
 65                  70                  75

Glu Leu Leu Glu Glu His Gly Glu Arg Lys Glu Glu Gly Cys Thr
             80                  85                  90

Gln Glu Asn Thr Glu Ser Glu Tyr Tyr Ala Lys Glu Ile His Lys
         95                 100                 105

Phe Asp Met Ile Gln Gly Leu Ala Glu His Asn Glu Leu Ala Val
    110                 115                 120

Cys Pro Lys Gly Ile Thr Ser Lys Val Phe Arg Phe Asn Val Ser
    125                 130                 135

Ser Val Glu Lys Asn Arg Thr Asn Leu Phe Arg Ala Glu Phe Arg
    140                 145                 150

Val Leu Arg Val Pro Asn Pro Ser Ser Lys Arg Asn Glu Gln Arg
    155                 160                 165

Ile Glu Leu Phe Gln Ile Leu Arg Pro Asp Glu His Ile Ala Lys
    170                 175                 180

Gln Arg Tyr Ile Gly Gly Lys Asn Leu Pro Thr Arg Gly Thr Ala
    185                 190                 195

Glu Trp Leu Ser Phe Asp Val Thr Asp Thr Val Arg Glu Trp Leu
    200                 205                 210

Leu Arg Arg Glu Ser Asn Leu Gly Leu Glu Ile Ser Ile His Cys
    215                 220                 225
```

Pro Cys His Thr Phe Gln Pro Asn Gly Asp Ile Leu Glu Asn Ile
230 235 240

His Glu Val Met Glu Ile Lys Phe Lys Gly Val Asp Asn Glu Asp
245 250 255

Asp His Gly Arg Gly Asp Leu Gly Arg Leu Lys Lys Gln Lys Asp
260 265 270

His His Asn Pro His Leu Ile Leu Met Met Ile Pro Pro His Arg
275 280 285

Leu Asp Asn Pro Gly Gln Gly Gly Gln Arg Lys Lys Arg Ala Leu
290 295 300

Asp Thr Asn Tyr Cys Phe Arg Asn Leu Glu Glu Asn Cys Cys Val
305 310 315

Arg Pro Leu Tyr Ile Asp Phe Arg Gln Asp Leu Gly Trp Lys Trp
320 325 330

Val His Glu Pro Lys Gly Tyr Tyr Ala Asn Phe Cys Ser Gly Pro
335 340 345

Cys Pro Tyr Leu Arg Ser Ala Asp Thr Thr His Ser Thr Val Leu
350 355 360

Gly Leu Tyr Asn Thr Leu Asn Pro Glu Ala Ser Ala Ser Pro Cys
365 370 375

Cys Val Pro Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Val
380 385 390

Gly Arg Thr Pro Lys Val Glu Gln Leu Ser Asn Met Val Val Lys
395 400 405

Ser Cys Lys Cys Ser
410

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 304 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(iv) SEQUENCE DESCRIPTION:SEQ ID NO:4:

Met Asp Pro Met Ser Ile Gly Pro Lys Ser Cys Gly Gly Ser Pro
1 5 10 15

Trp Arg Pro Pro Gly Thr Ala Pro Trp Ser Ile Gly Ser Arg Arg
20 25 30

Ala Thr Ala Ser Ser Ser Cys Ser Thr Ser Ser Arg Val Arg Ala
35 40 45

Glu Val Gly Gly Arg Ala Leu Leu His Arg Ala Glu Leu Arg Met
50 55 60

Leu Arg Gln Lys Ala Ala Ala Asp Ser Ala Gly Thr Glu Gln Arg
65 70 75

Leu Glu Leu Tyr Gln Gly Tyr Gly Asn Ala Ser Trp Arg Tyr Leu
80 85 90

His Gly Arg Ser Val Arg Ala Thr Ala Asp Asp Glu Trp Leu Ser
95 100 105

Phe Asp Val Thr Asp Ala Val His Gln Trp Leu Ser Gly Ser Glu
110 115 120

Leu Leu Gly Val Phe Lys Leu Ser Val His Cys Pro Cys Glu Met
125 130 135

Gly Pro Gly His Ala Asp Glu Met Arg Ile Ser Ile Glu Gly Phe
140 145 150

Glu Gln Gln Arg Gly Asp Met Gln Ser Ile Ala Lys Lys His Arg
155 160 165

Arg Val Pro Tyr Val Leu Ala Met Ala Leu Pro Ala Glu Arg Ala
170 175 180

Asn Glu Leu His Ser Ala Arg Arg Arg Arg Asp Leu Asp Thr Asp
185 190 195

Tyr Cys Phe Gly Pro Gly Thr Asp Glu Lys Asn Cys Cys Val Arg
200 205 210

Pro Leu Tyr Ile Asp Phe Arg Lys Asp Leu Gln Trp Lys Trp Ile
215 220 225

His Glu Pro Lys Gly Tyr Met Ala Asn Phe Cys Met Gly Pro Cys
230 235 240

Pro Tyr Ile Trp Ser Ala Asp Thr Gln Tyr Thr Lys Val Leu Ala
245 250 255

Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala Pro Cys Cys
260 265 270

Val Pro Gln Thr Leu Asp Pro Leu Pro Ile Ile Tyr Tyr Val Gly
275 280 285

Arg Asn Val Arg Val Glu Gln Leu Ser Asn Met Val Val Arg Ala
290 295 300

Cys Lys Cys Ser
304

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 198 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(iv) SEQUENCE DESCRIPTION:SEQ ID NO:5:

```
Asp Glu Trp Met Ser Phe Asp Val Thr Lys Thr Val Asn Glu Trp
  1               5                  10                  15

Leu Lys Arg Ala Glu Glu Asn Glu Gln Phe Gly Leu Gln Pro Ala
             20                  25                  30

Cys Lys Cys Pro Thr Pro Gln Ala Lys Asp Ile Asp Ile Glu Gly
         35                  40                  45

Phe Pro Ala Leu Arg Gly Asp Leu Ala Ser Leu Ser Ser Lys Glu
     50                  55                  60

Asn Thr Lys Pro Tyr Leu Met Ile Thr Ser Met Pro Ala Glu Arg
 65                  70                  75

Ile Asp Thr Val Thr Ser Ser Arg Lys Lys Arg Gly Val Gly Gln
             80                  85                  90

Glu Tyr Cys Phe Gly Asn Asn Gly Pro Asn Cys Cys Val Lys Pro
         95                 100                 105

Leu Tyr Ile Asn Phe Arg Lys Asp Leu Gly Trp Lys Trp Ile His
        110                 115                 120

Glu Pro Lys Gly Tyr Glu Ala Asn Tyr Cys Leu Gly Asn Cys Pro
    125                 130                 135

Tyr Ile Trp Ser Met Asp Thr Gln Tyr Ser Lys Val Leu Ser Leu
140                 145                 150

Tyr Asn Gln Asn Asn Pro Gly Ala Ser Ile Ser Pro Cys Cys Val
155                 160                 165

Pro Asp Val Leu Glu Pro Leu Pro Ile Ile Tyr Tyr Val Gly Arg
            170                 175                 180

Thr Ala Lys Val Glu Gln Leu Ser Asn Met Val Val Arg Ser Cys
        185                 190                 195

Asn Cys Ser
    198
```

Claims

1. A method of predisposing a mammal to accelerated tissue repair comprising systemically administering to the mammal, prior to its exposure to tissue damage, an effective amount of TGF-β.

2. The method of claim 1 wherein the TGF-β is administered no more than about 24 hours prior to the tissue damage exposure.

3. The method of claim 1 wherein the TGF-β is administered within a time range of from about 24 hours to greater than about 5 minutes prior to the tissue damage exposure.

4. The method of claim 1 wherein the administration is in a single dose.

5. The method of claim 1 wherein the administration is intravenous.

6. The method of claim 1 wherein the TGF-β is human TGF-β.

7. The method of claim 6 wherein the TGF-β is TGF-β1.

8. The method of claim 1 wherein the mammal is human.

9. The method of claim 1 wherein the tissue repair is wound healing and the tissue damage is a wound.

10. The method of claim 1 wherein the tissue repair is bone repair and the tissue damage is a bone fracture, prosthetic implant, or bony defect.

11. The method of claim 1 wherein the tissue damage is surgical incision.

```
                1         10         20              32        40          50
Hu TGF-β  1 MPPSGLRLLPLLLPLLWLLV-LTPGPPAAGLSTCKTIDMELVKRKRIEAIR
Hu TGF-β  2 MHYCVLSAFLILHLV---TVAL------S-LSTCSTLDMDQFMRKRIEAIR
Hu TGF-β  3 --MHLQRALVVLALLNFATVSL------S-LSTCTTLDFGHIKKKRVEAIR

                       60          70         80         90
Hu TGF-β  1 GQILSKLRLASPPSQGE-VP-PGPLPEAVLALYNSTRDRVAGESAEPE-PE
Hu TGF-β  2 GQILSKLKLTSPP---EDYPEPEEVPPEVISIYNSTRDLL--QEKASR-RA
Hu TGF-β  3 GQILSKLRLTSPP---EPTV-MTHVPYQVLALYNSTRELL--EEHGER-KE
Ck TGF-β  4 ------------------------------------M--DPMSIGPK-

                    100        110             120         130
Hu TGF-β  1 P-------EADYYAKEVTRVLMV----ETHNEIYDKFKQSTHSIYMFFNTS
Hu TGF-β  2 AACERERSDEEYYAKEVYKIDMPPFFPS-EHAIPPTFYRPY-FRIVRFDVS
Hu TGF-β  3 EGCTQENTESEYYAKEIHKFDMIQGLAE-HNELAVCPKGIT-SKVFRFNVS
Ck TGF-β  4 -SCG--------------------GSPW-RPP-GTAPWSIG-SR--RATAS

                  140             150         160             170
Hu TGF-β  1 EL-----RE-AVPEPVLLS-RAELRLLRLKL----KV-EQHVELYQ-----
Hu TGF-β  2 A---------MEKNASNLV-KAEFRVFRLQNPK-ARVPEQRIELYQILKSK
Hu TGF-β  3 S---------VEKNRTNLF-RAEFRVLRVPNPS-SKRNEQRIELFQILRP-
Ck TGF-β  4 SSCSTSSRVRAEVGGRALLHRAELRMLRQKAAADSAGTEQRLELYQGYGN-

                    180        190        200        210        220
Hu TGF-β  1 KYSNNSWRYLSNRLLAPSDSPEWLSFDVTGVVRQWLSRGGEIEGFRLSAHC
Hu TGF-β  2 DLTSPTQRYIDSKVVKTRAEGEWLSFDVTDAVHEWLHHKDRNLGFKISLHC
Hu TGF-β  3 DEHIAKQRYIGGKNLPTRGTAEWLSFDVTDTVREWLLRRESNLGLEISIHC
Ck TGF-β  4 ----ASWRYLHGRSVRATADDEWLSFDVTDAVHQWLSGSELLGVFKLSVHC
Fg TGF-β  5 --------------------DEWMSFDVTKTVNEWLKRAEENEQFGLQPAC
```

FIG. 1A

```
                              230         240              250
Hu TGF-β 1 SC---------DSRDNTLQVDIN-GFTTGR------RGDLATI---------
Hu TGF-β 2 PCCTFVPSNNYIIPNKSEELEARFA-GIDGTSTYTSGDQKTIKSTRKKNSG
Hu TGF-β 3 PCHTFQP-NGDILENIHEVMEIKFK-GVDNEDDHGRGDLGRLK---KQKDH
Ck TGF-β 4 PCEMGPG-HADEMRISIEGFEQQ------------RGDMQSIA---K-KHR
Fg TGF-β 5 KCPT-PQ-AKDI-D--IEGFPAL------------RGDLASLSS--KEN--

                         260         270              280          290
Hu TGF-β 1 HGMNRPFLLLMATPLERA-QH--LQSS---RHRRALDTNYCF--SSTEKNC
Hu TGF-β 2 KT---PHLLLMLLPSYRL-ESQ----QTNRRKKRALDAAYCF--RNVQDNC
Hu TGF-β 3 H--N-PHLILMMIPPHRL-DNPGQGGQ---RKKRALDTNYCF--RNLEENC
Ck TGF-β 4 R--V-PYVLAMALPAERANE---LHSA---RRRRDLDTDYCFGPGTDEKNC
Fg TGF-β 5 -TK--PYL--MIT-SMPAERIDTVTSS---RKKRGVGQEYCF--GNNGPNC

                      300         310         320         330         340
Hu TGF-β 1 CVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYN
Hu TGF-β 2 CLRPLYIDFKRDLGWKWIHEPKGYNANFCAGACPYLWSSDTQHSRVLSLYN
Hu TGF-β 3 CVRPLYIDFRQDLGWKWVHEPKGYYANFCSGPCPYLRSADTTHSTVLGLYN
Ck TGF-β 4 CVRPLYIDFRKDLQWKWIHEPKGYMANFCMGPCPYIWSADTQYTKVLALYN
Fg TGF-β 5 CVKPLYINFRKDLGWKWIHEPKGYEANYCLGNCPYIWSMDTQYSKVLSLYN

                      350         360         370         380         390
Hu TGF-β 1 QHNPGASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS
Hu TGF-β 2 TINPEASASPCCVSQDLEPLTILYYIGKTPKIEQLSNMIVKSCKCS
Hu TGF-β 3 TLNPEASASPCCVPQDLEPLTILYYVGRTPKVEQLSNMVVKSCKCS
Ck TGF-β 4 QHNPGASAAPCCVPQTLDPLPIIYYVGRNVRVEQLSNMVVRACKCS
Fg TGF-β 5 QNNPGASISPCCVPDVLEPLPIIYYVGRTAKVEQLSNMVVRSCNCS
```

FIG. 1B

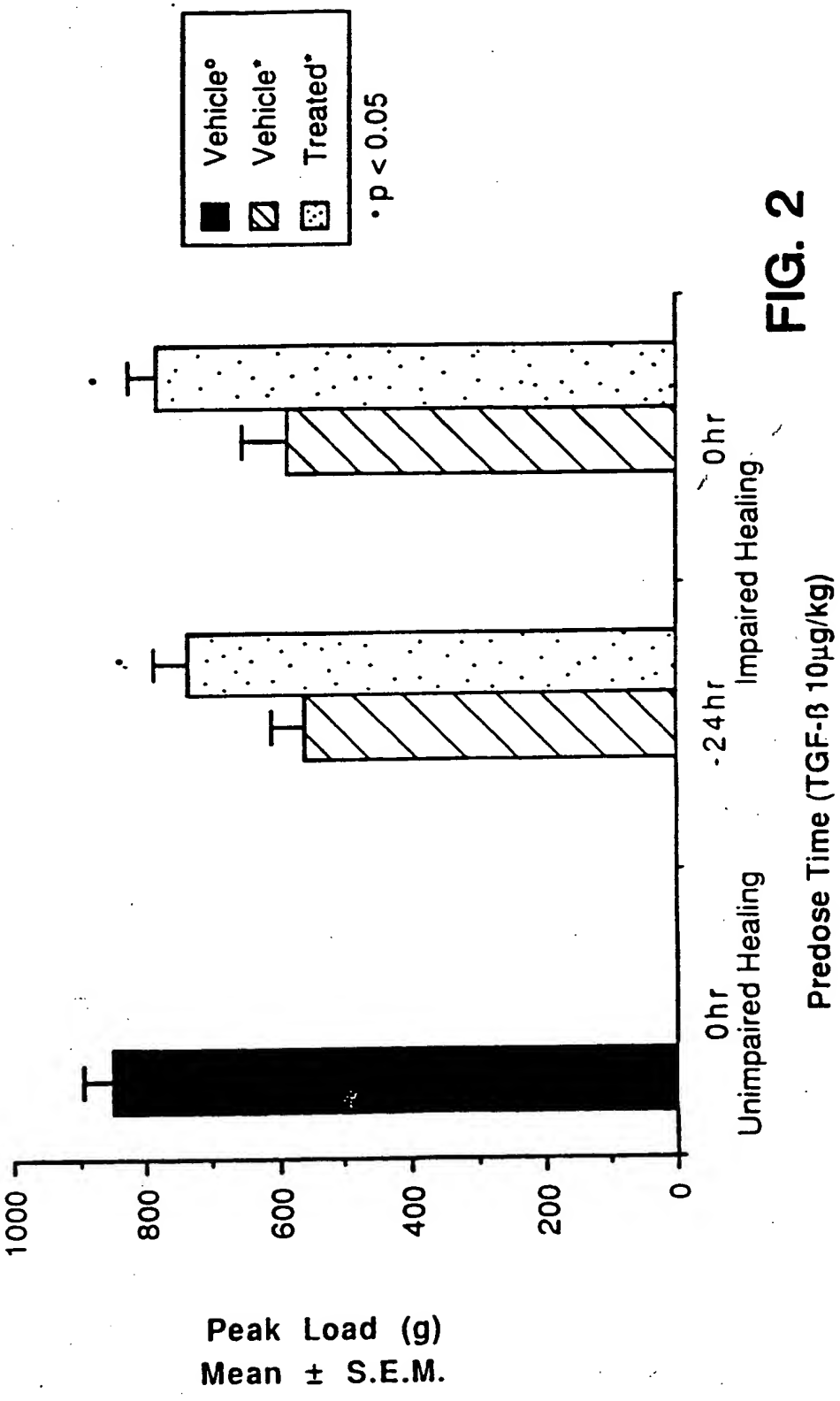

Vehicle*
Vehicle*
Treated*
* p < 0.05
0hr
Unimpaired Healing
24hr
Impaired Healing
0hr
Predose Time (TGF-β 10μg/kg)
1000
800
600
400
200
0
Peak Load (g)
Mean ± S.E.M.

FIG. 2

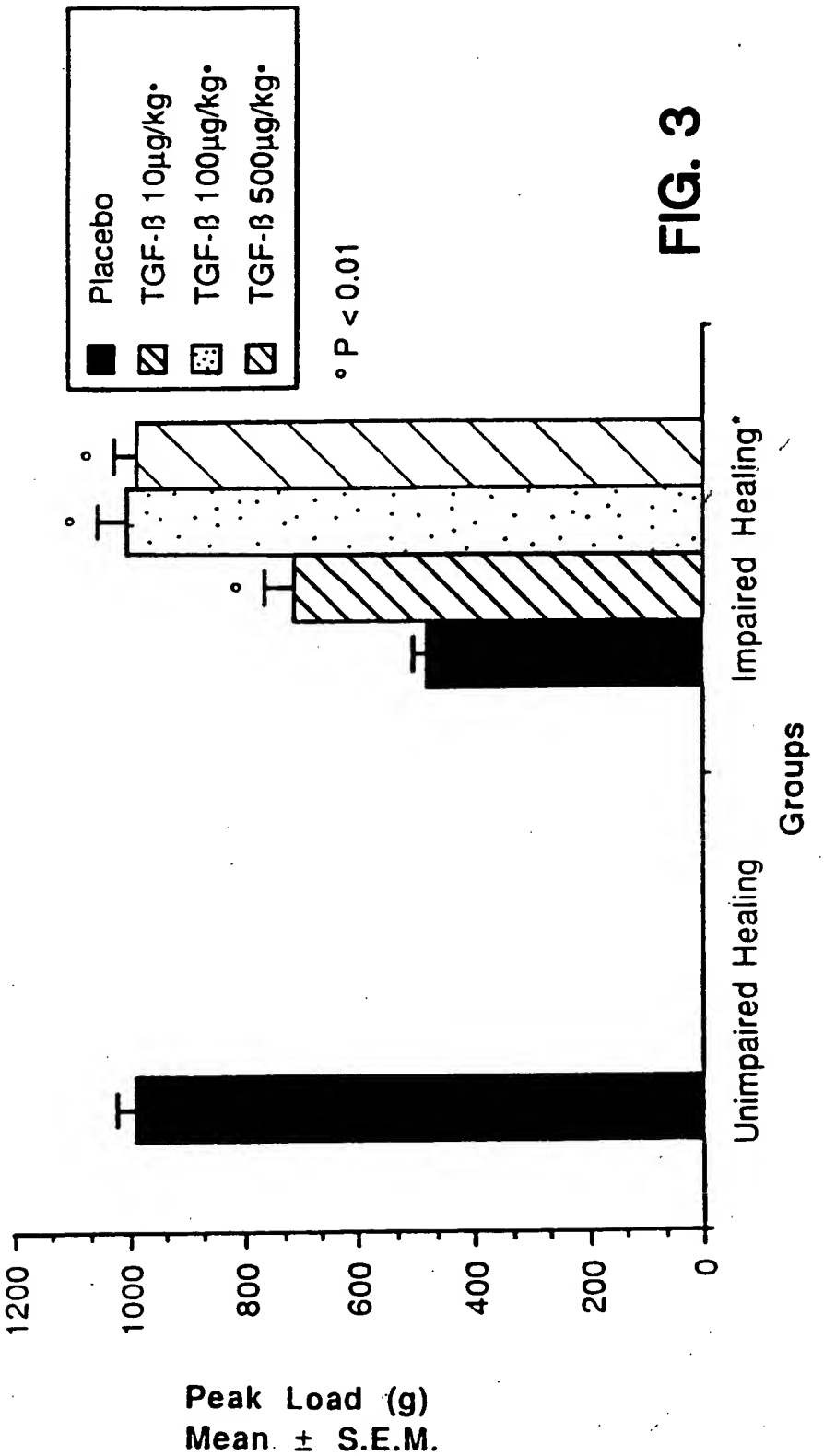

Placebo
TGF-ß 10µg/kg•
TGF-ß 100µg/kg•
TGF-ß 500µg/kg•
° P < 0.01
°
°
°
1200
1000
800
600
400
200
0
Peak Load (g)
Mean ± S.E.M.
Unimpaired Healing
Impaired Healing•
Groups

FIG. 3

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.